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(57) Abstract

A process for improving the treatment of a tumor by radiation therapy which comprises treating a tumor by radiation therapy wherein the cells of the tumor have been transduced with a polynucleotide encoding wild-type p53, such as, for example, by transducing the tumor cells with an adenoviral vector including a DNA sequence encoding wild-type p53. Such a combination treatment of the transduction of tumor cells with a polynucleotide encoding wild-type p53 and radiation therapy provides a more effective treatment than by using p53 gene therapy alone or radiation therapy alone.

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CANCER TREATMENT

This application is a continuation-in-part of Application Serial No. 08/527,373, filed September 13, 1995, the contents of which are incorporated herein by reference.

This invention relates to cancer treatment and more particularly to the treatment of cancer by gene therapy. Still more particularly, the invention relates to treatment of cancer by a combination of gene therapy and radiation therapy.

BACKGROUND OF THE INVENTION

Cancer is a devastating disease, and there is a need to find new methods for treatment, particularly for those cancers which have been particularly difficult to treat.

For example, each year in the U.S. approximately 40,000 individuals will be diagnosed with squamous cell carcinoma of the head and neck (SCCHN) and upper aerodigestive tract. This disease not only has a profound effect upon speech, swallowing and physical appearance, but has an overall survival rate of only approximately 50%, a rate which has remained relatively unchanged for more than thirty years. Treatment often requires aggressive adjunctive therapy after surgery, with radiation being the most common form. However, a significant number (30-40%) of squamous cell carcinomas of the head and neck have been found to be resistant to radiotherapy. Failure to respond to radiation

therapy has been an unmet medical need in the treatment of head and neck tumors and other forms of cancer as well. The identification of an improved therapy would have immense clinical significance.

Squamous cell carcinomas of the head and neck area (SCCHN) arise from a multiplicity of sites and are primarily due to environmental factors, principally, the use of alcohol and tobacco.

These tumors are notoriously difficult to treat with a high percentage of recurrence. One of the major problems is the presence of microscopic residual tumor after surgery at the site of the primary tumor. Despite advances in current treatments, patients with advanced disease have a poor prognosis. More than two thirds of the individuals in whom the diagnosis of squamous cell carcinoma of the head and neck is made will present with state III or IV disease at the primary and/or nodal sites. Despite optimal local therapy, 50%-60% of these patients will ultimately develop local recurrence, and 30% or more will develop distant metastatic disease. The overall survival rate is 40% for patients whose tumors are completely resected and 20% for those with unresectable tumors treated with radiotherapy alone. Treatment of recurrent disease is palliative at best and without long-term benefit. Furthermore, the long-term outlook for those surviving their first malignancy is overshadowed by a 10% to 40% rate of a second primary malignancy.

Incorporation of chemotherapy into the early treatment regimen of locally advanced disease has reduced morbidity by increasing organ preservation. However, as the majority of those receiving chemotherapy still develop recurrence of the primary disease, its role in increasing overall survival remains to be demonstrated. Historically, surgery has been the primary form of treatment, with the addition of radiotherapy in advanced stages in an effort to reduce

recurrence. Recently, combined modality approaches employing chemotherapy, joint chemoradiotherapy or alternating chemo and radio therapies have been tried. However, only a few of the studies, those using simultaneous chemo-radio treatment, were able to demonstrate increased survival. Stupp, *et al.*, Seminars in Oncology, Vol. 21, pgs. 349-358 (1994).

Since the early part of the century radiation has been the treatment of choice for most head and neck cancers and remains an integral part, either as a single modality or as a part of a multimodal course, of treatment for this disease. The most commonly used radioisotopes employed in radiotherapy are X and gamma rays (photon radiation). Additionally, charged (electrons, protons, and heavy ions such as helium and neon) and uncharged particles (neutrons) can also be used in radiation treatment.

Standard radiation therapy in the U.S. consists of five daily (fractionated) doses of 1.8 to 2.25 Gy per week given continuously for five to seven weeks. The total dose is dependent upon the size of the tumor, its histology and the normal tissue tolerance. Gross visible SCCHN tumors usually require total doses of 65 to 75 Gy while microscopic disease usually receives 45 to 50 Gy over 4-1/2 to 5-1/2 weeks. The extent of treatment is also dependent on the site and stage of the disease (reviewed in Awan, *et al.*, Hematology/Oncology Clinics of North America, Vol. 5, pgs. 635-655 (1991)). Several non-standard patterns of treatment have also been examined (Awan, *et al.*, 1991): 1) Hyperfractionation, where smaller doses are given more than once per day over the same time span as with standard treatment; 2) Accelerated Fractionation, where multiple daily doses are given resulting in shortening of overall treatment time; 3) Split-course, where standard doses are given but with a break midway in the treatment course; and 4) Hypofractionation, which is usually one or two large doses given weekly for several weeks. Many of the non-standard regimens being

employed may be a combination of one or more of the four described above. (Awan, *et al.*, 1991).

Many factors contribute to the control of head and neck cancer. Besides the growth characteristics and the number of cells in the tumor, various aspects of the tumor microenvironment, including the pH, reoxygenation, and hypoxia are important. Also to be considered is the inherent radioresistance/sensitivity of the tumor cells themselves. However, the genetic basis of ionizing radiation resistance (RR) in mammalian cells is poorly understood. The individual molecular events and specific genes involved will affect both normal cellular protection from radiation damage as well as failure of tumors to respond to radiation therapy.

P53 may also play a role in the development and progression of SCCHN. Depending upon the tissue source and method of detection of abnormal p53, both the gene and its expression have been identified in 33% to 100% of head and neck cancers. Mutations have been found in exons 4 through 9 with a hot spot in the codon 238-248 region (Field, *et al.*, Arch. Otolaryngol. Head and Neck Surg., Vol. 119, pgs. 1118-1122 (1993); Brachman, Seminars in Oncology, Vol. 21, pgs. 320-329 (1994)). Moreover, preliminary studies indicate that the presence of p53 mutations may be indicative of a higher frequency and shorter median time to recurrence of the tumor (Brachman, 1994). Abnormal p53 also correlates with a history of heavy smoking and drinking which are the primary environmental factors associated with SCCHN (Field, *et al.*, 1993).

Studies indicate that the presence of p53 mutations may also be indicative in SCCHN of a higher frequency of, and shorter median time to, recurrence of the tumor (Brachman, Seminars in Oncology, Vol. 21, pgs. 320-329 (1994)). In a recent study it was reported that higher levels of mutated p53 expression in primary SCCHN, due presumably to the stabilization and longer half-life of the mutant form of the

protein, were associated with both earlier recurrence and development of second primary tumors and could be an important adverse prognostic factor for survival (Shin, *et al.*, J. Nat. Cancer Inst., Vol. 88, pgs. 519-529 (1996)).

A role for wild type p53 in the control of cellular proliferation by induction of programmed cell death (apoptosis) (Lowe, *et al.*, Nature, Vol. 362, pp. 847-848 (1993); Clarke, *et al.*, Nature, Vol. 362, pgs. 849-852 (1993); Youish-Rouach, *et al.*, Nature, Vol. 353, pgs. 345-347 (1991); Lowe, *et al.*, Science, Vol. 266, pgs. 807-810 (1994)) and the regulation of cell cycle events (Kastan, *et al.*, Cancer Research, Vol. 51, pgs. 6304-6311 (1991); Kuerbitz, *et al.*, Proc. Nat. Acad. Sci., Vol. 89, pgs. 7491-7495 (1992); Kastan, *et al.*, Cell, Vol. 7, pgs. 587-597 (1992)) has been established. Furthermore, investigators using p53 knockout mice which lack wild type p53 have demonstrated that thymocytes from these animals are resistant to gamma-radiation, or etoposide (a toposisomerase II inhibitor) induced apoptosis, whereas thymocytes from control mice with wild type p53 showed radiosensitivity to gamma-radiation (Lowe, *et al.*, 1993; Clarke, *et al.*, 1993).

Although, Kastan, *et al.*, Cancer Research, Vol. 51, pgs. 6304-6311 (December 1, 1991), discloses that p53 expression increases after treatment of ML-1 myeloid leukemia cells with gamma-radiation, whereby such cells remain in G_1 arrest, Jung, *et al.*, Cancer Research, Vol. 52, pgs. 6390-6393 (1992) disclose mutations in the p53 gene in the SCC-35, JSQ-3, SQ-38, SCC-9, and SCC-9G squamous carcinoma cell lines. The SCC-35 and JSQ-3 cell lines are radiation resistant, while the SQ-38, SCC-9 and SCC-9G cell lines are radiation sensitive.

The correlation between p53 and apoptosis, in combination with the apparently normal development of mice lacking wild type p53 (Donehower, *et al.*, Nature, Vol. 356, pgs. 215-221 (1992)), and the observations of a post-

radiation G1 block, suggests that wild type p53 functions in the regulation of the cell after DNA damage or stress rather than during proliferation and development. As the presence of mutated p53 also has been shown to correlate with increased radiation resistance in some human tumors and cell lines (Agarwal, *et al.*, Proc. Nat. Acad. Sci., Vol. 92, pgs. 8493-8497 (1995); Donehower, *et al.*, 1992; Lee, *et al.*, Proc. Nat. Acad. Sci., Vol. 90, pgs. 5742-5746 (1993); O'Conner, *et al.*, Cancer Research, Vol. 53, pgs. 4776-4780 (1993); McIlwrath, *et al.*, Cancer Research, Vol. 54, pgs. 3718-3722 (1994)) and a high percentage of head and neck tumors fail radiation therapy, a cause and effect relationship may exist between the lack of functional wild type p53 found in a large number of squamous cell carcinomas of the head and neck and radiation resistance. Thus, the replacement of wild type p53 may ameliorate the high level of radiation resistance frequently observed in these cancers.

SUMMARY OF THE INVENTION

The present invention is directed to the treatment of tumors whereby such tumors are treated with a combination of radiation therapy and transduction with a polynucleotide encoding wild type p53. Such treatment may be employed in treating radiation resistant tumors as well as radiation-sensitive tumors.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention now will be described with respect to the drawings, wherein:

Figure 1 is a map of plasmid pAvS6;

Figure 2 is a map of plasmid pAvS6-nLacZ;

Figure 3 is a schematic of the construction of Av1LacZ4;

Figure 4 is a schematic of the construction of pAvS6.p53;

Figure 5 is a schematic of the construction of Av1p53;

Figure 6 depicts growth inhibition of tumor cell lines by wild type p53 in the SCCHN cell lines JSQ-3, SQ-20B, and

SCC 61; the ovarian cancer cell line SK-OV-3; the breast cancer cell line SK-BR-3; and the normal skin fibroblast cell line H500. The cells were treated with increasing doses of Av1p53 or Av1LacZ4. Two virus doses were given 24 hours apart. Four days after the second treatment, the cells were stained with Giemsa stain. MOI=multiplicity of infection;

Figure 7 shows light micrographs of JSQ-3 cells which were treated with Av1p53 or Av1Lacz4, or were untreated, magnification 200 X. Panel A - untreated cells; Panel B - cells after treatment with 10 MOI of Av1p53; Panel C - cells after treatment with 20 MOI of Av1p53; Panel D - cells after treatment with 40 MOI of Av1p53; Panel E - cells after treatment with 80 MOI of Av1p53; Panel F - cells after treatment with 160 MOI of Av1p53; Panel G - cells after treatment with 80 MOI of Av1LacZ4; and Panel H - cells after treatment with 320 MOI of Av1LacZ4.

Figure 8 is a graph of the D_{10} values (Gy) of JSQ-3 cells at 24 and 36 hours after treatment with 5, 10, or 20 MOI of Av1p53 or 20 MOI of Av1LacZ4;

Figure 9 show graphs of survival curves, after graded doses of gamma-radiation, for JSQ-3 cells treated for 24 or 36 hours with 5, 10, or 20 MOI of Av1p53 or 20 MOI of Av1LacZ4. Curves are plotted as the logarithm of the surviving fraction versus radiation dose. Points are plotted as the mean ± SE of 2 to 9 experiments;

Figure 10 is a Western blot analysis of p53 expression in JSQ-3 cells transduced with increasing doses of Av1p53. p53 protein expression was examined thirty-six hours after viral infection. C represents uninfected JSQ-3 cells. The amount of Av1p53 for viral infection is given as multiplicity of infection (MOI);

Figure 11 is a graph of the mean tumor volumes in mice injected with JSQ-3 cells followed by radiation treatment and treatment with 10 MOI Av1p53 or 10 MOI Av1LacZ4;

Figure 12 is a graph of the mean tumor volumes in mice injected with JSQ-3 cells followed by no treatment, radiation treatment alone, Av1p53 treatment alone, or radiation treatment combined with treatment with Av1p53 or Av1LacZ4.

Figure 13 depicts graphs of the effect of the combination of wild type p53 replacement and radiation therapy on tumor growth in a xenograft mouse model. Tumor size was measured before viral injection with either Av1p53 or Av1LacZ4 (Day 0), before each radiation dose, and weekly thereafter. Data are calculated as percent of original tumor volume, and plotted as fractional tumor volume [f (s/so)] ±SE. V represents the day of viral injection. R represents eight 2.5 Gy doses of radiation, for a total of 20 Gy;

Figure 14 depicts light micrographs of histochemical analyses of subcutaneous xenograft tumors before and after treatment with radiation and/or Av1p53. Subcutaneous tumors were excised from the animals before or after treatment with radiation and/or Av1p53 or the control vector Av1LacZ4. Panel A - control tumor, untreated, non-irradiated; Panel B- tumor after 2 injections of 1 x 10^8 pfu of Av1p53, without radiation; Panel C - tumor after 20 Gy of ionizing radiation, without viral treatment; Panel D - tumor after 2 injections of 1 x 10^8 pfu of control vector Av1LacZ4 plus 20 Gy of ionizing radiation; Panel E - tumor after a combination of 20 Gy of ionizing radiation plus 2 injections of 5 x 10^7 pfu of Av1p53; Panel F - tumor after a combination of 20 Gy of ionizing radiation plus 2 injections of 1 x 10^8 pfu of Av1p53. Magnification 400X;

Figures 15A and 15B are graphs of the percentages of JSQ-3 cells and H500 cells, respectively, in the G1, S, or G2+M phases of the cell cycle at 24 to 48 hours after exposure to 8 Gy of gamma-radiation, as determined by FACS analysis (-) V/(-)R represents non-virally infected, non-irradiated cells; (-)V/(+)R represents non-virally infected, radiated cells; (+)V/(-)R represents cells infected with 30

MOI of Av1p53 but not irradiated; and (+)V/(+)R represents cells infected with 30 MOI of Av1p53 prior to radiation;

Figure 16 is a graph of the percentages of apoptotic cells as determined by FACS analysis, in a JSQ-3 cell population, at 72 hours after exposure to 8 Gy of gamma-radiation. (-)V/(-)R represents non-virally infected, non-irradiated cells; (-)V/(+)R represents non-virally infected, irradiated cells; (+)V/(-)R represents cells infected with 30 MOI of Av1p53 but not irradiated; and (+)V/(+)R represents cells infected with 30 MOI of Av1p53 at 36 hours prior to radiation; and

Figure 17 is a graph of the effect of the combination of wild type p53 replacement and radiation therapy on tumor growth in a xenograft mouse model. The tumor size was measured before viral injection with either Av1p53 or Av1LacZ4 (Day 0), before each radiation dose, and weekly thereafter. Data are calculated as mean tumor volumes of 7 to 9 mice. V represents the day of vial injection; R represents eight 2.5 Gy radiation doses, for a total of 20 Gy; Cell (-)R represents control, non-radiated tumors; Cell (+)R represents non-virally infected, irradiated tumors; L5(+)R represents irradiated tumors injected with 5×10^8 pfu of Av1LacZ4; L20(+)R represents irradiated tumors injected with 2×10^9 pfu of Av1LacZ4; P5(+)R represents irradiated tumors injected with 5×10^8 pfu of Av1p53; P10(+)R represent irradiated tumors injected with 1×10^9 pfu of Av1p53; and P20(+)R represents irradiated tumors injected with 2×10^9 pfu of Av1p53.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with one aspect of the present invention, there is provided a process for treating cancer, and in particular squamous cell carcinoma of the head and neck and upper aerodigestive tract, by a combination of gene therapy and radiation therapy.

More particularly, the present invention is directed to cancer treatment wherein tumor cells are transduced or transfected with a polynucleotide encoding wild type p53 protein in conjunction with irradiation of the tumor.

Applicants have found that such combination results in a more effective treatment than using p53 gene therapy alone or radiation therapy alone. Applicants also have found that such treatment enhances the radiation sensitivity of tumor cells, whether such tumor cells normally are radiation resistant or radiation sensitive. Applicants have found that the transduction of radiation resistant tumor cells with a polynucleotide encoding wild type p53 can reverse the radiation resistance of such tumor cells. Thus, the treatment of the present invention may be employed in treating radiation resistant tumors as well as radiation sensitive tumors.

Thus, in accordance with an aspect of the present invention, there is provided a process for treating radiation resistant tumors wherein the effect of radiation therapy is enhanced by providing cells of the tumor with a polynucleotide encoding wild type p53.

The term "treating a tumor" as used herein means that one provides for the inhibition, prevention, or destruction of the growth of the tumor cells.

The term "polynucleotide" as used herein means a polymeric form of nucleotide of any length, and includes ribonucleotides and deoxyribonucleotides. Such term also includes single- and double-stranded DNA, as well as single- and double-stranded RNA. The term also includes modified polynucleotides such as methylated or capped polynucleotides.

The gene encoding wild-type p53 is obtainable through sources known to those skilled in the art (*e.g.*, Genbank, ATCC, etc.), and/or may be isolated from expression vehicles (*e.g.*, plasmids) obtainable through sources known to those

skilled in the art through standard techniques (*e.g.*, PCR) known to those skilled in the art.

The polynucleotide encoding wild-type p53 may be contained within an appropriate expression vehicle which has been transduced into the cell. Such expression vehicles include, but are not limited to, plasmids, eukaryotic vectors, prokaryotic vectors (such as, for example, bacterial vectors), and viral vectors.

In one embodiment, the vector is a viral vector. Viral vectors which may be employed include RNA virus vectors (such as retroviral vectors), and DNA virus vectors (such as adenoviral vectors, adeno-associated virus vectors, Herpes Virus vectors, and vaccinia virus vectors). When an RNA virus vector is employed, in constructing the vector, the polynucleotide encoding wild-type p53 is in the form of RNA. When a DNA virus vector is employed, in constructing the vector, the polynucleotide encoding wild-type p53 is in the form of DNA.

In a preferred embodiment, the viral vector is an adenoviral vector.

The adenoviral vector which is employed may, in one embodiment, be an adenoviral vector which includes essentially the complete adenoviral genome (Shenk *et al.*, Curr. Top. Microbiol. Immunol., 111(3): 1-39 (1984). Alternatively, the adenoviral vector may be a modified adenoviral vector in which at least a portion of the adenoviral genome has been deleted.

In the preferred embodiment, the adenoviral vector comprises an adenoviral 5' ITR; an adenoviral 3' ITR; an adenoviral encapsidation signal; a DNA sequence encoding wild-type p53; and a promoter controlling the DNA sequence encoding wild-type p53. The vector is free of at least the majority of adenoviral E1 and E3 DNA sequences, but is not free of all of the E2 and E4 DNA sequences, and DNA sequences

encoding adenoviral proteins promoted by the adenoviral major late promoter.

In one embodiment, the vector also is free of at least a portion of at least one DNA sequence selected from the group consisting of the E2 and E4 DNA sequences.

In another embodiment, the vector is free of at least the majority of the adenoviral E1 and E3 DNA sequences, and is free of a portion of the other of the E2 and E4 DNA sequences.

In still another embodiment, the gene in the E2a region that encodes the 72 kilodalton binding protein is mutated to produce a temperature sensitive protein that is active at 32°C, the temperature at which the viral particles are produced. This temperature sensitive mutant is described in Ensinger *et al.*, J. Virology, 10:328-339 (1972), Van der Vliet *et al.*, J. Virology, 15:348-354 (1975), and Friefeld *et al.*, Virology, 124:380-389 (1983).

Such a vector, in a preferred embodiment, is constructed first by constructing, according to standard techniques, a shuttle plasmid which contains, beginning at the 5' end, the "critical left end elements," which include an adenoviral 5' ITR, an adenoviral encapsidation signal, and an E1a enhancer sequence; a promoter (which may be an adenoviral promoter or a foreign promoter); a multiple cloning site (which may be as herein described); a poly A signal; and a DNA segment which corresponds to a segment of the adenoviral genome. The vector also may contain a tripartite leader sequence. The DNA segment corresponding to the adenoviral genome serves as a substrate for homologous recombination with a modified or mutated adenovirus, and such sequence may encompass, for example, a segment of the adenovirus 5 genome no longer than from base 3329 to base 6246 of the genome. The plasmid may also include a selectable marker and an origin of replication. The origin of replication may be a bacterial origin of replication. Representative examples of such

shuttle plasmids include pAvS6, which is described in published PCT Application Nos. W094/23582, published October 27, 1994, and W095/09654, published April 13, 1995. The DNA sequence encoding wild-type p53 may then be inserted into the multiple cloning site to produce a plasmid vector.

This construct is then used to produce an adenoviral vector. Homologous recombination is effected with a modified or mutated adenovirus in which at least the majority of the E1 and E3 adenoviral DNA sequences have been deleted. Such homologous recombination may be effected through co-transfection of the plasmid vector and the modified adenovirus into a helper cell line, such as 293 cells, by $CaPO_4$ precipitation. Upon such homologous recombination, a recombinant adenoviral vector is formed that includes DNA sequences derived from the shuttle plasmid between the Not I site and the homologous recombination fragment, and DNA derived from the E1 and E3 deleted adenovirus between the homologous recombination fragment and the 3' ITR.

In one embodiment, the homologous recombination fragment overlaps with nucleotides 3329 to 6246 of the adenovirus 5 (ATCC VR-5) genome.

Through such homologous recombination, a vector is formed which includes an adenoviral 5' ITR, an adenoviral encapsidation signal; an E1a enhancer sequence; a promoter; a DNA sequence encoding wild-type p53 protein; a poly A signal; adenoviral DNA free of at least the majority of the E1 and E3 adenoviral DNA sequences; and an adenoviral 3' ITR. The vector also may include a tripartite leader sequence. The vector may then be transfected into a helper cell line, such as the 293 helper cell line (ATCC No. CRL1573), which will include the E1a and E1b DNA sequences, which are necessary for viral replication, and to generate adenoviral particles. Transfection may take place by electroporation, calcium phosphate precipitation, microinjection, or through proteoliposomes.

The vector hereinabove described may include a multiple cloning site to facilitate the insertion of the DNA sequence encoding the wild-type p53 into the cloning vector. In general, the multiple cloning site includes "rare" restriction enzyme sites; *i.e.*, sites which are found in eukaryotic genes at a frequency of from about one in every 10,000 to about one in every 100,000 base pairs. An appropriate vector is thus formed by cutting the cloning vector by standard techniques at appropriate restriction sites in the multiple cloning site, and then ligating the DNA sequence encoding wild-type p53 into the cloning vector.

The DNA sequence encoding wild-type p53 is under the control of a suitable promoter, which may be selected from those herein described, or such DNA may be under the control of its own native promoter.

In one embodiment, the adenovirus may be constructed by using a yeast artificial chromosome (or YAC) containing an adenoviral genome according to the method described in Ketner, *et al.*, PNAS, Vol. 91, pgs. 6186-6190 (1994), in conjunction with the teachings contained herein. In this embodiment, the adenovirus yeast artificial chromosome is produced by homologous recombination *in vivo* between adenoviral DNA and yeast artificial chromosome plasmid vectors carrying segments of the adenoviral left and right genomic termini. A DNA sequence encoding wild-type p53 then may be cloned into the adenoviral DNA. The modified adenoviral genome then is excised from the adenovirus yeast artificial chromosome in order to be used to generate adenoviral vector particles as hereinabove described.

The adenoviral vector particles are administered to an animal host in an amount which in combination with radiation therapy is effective to inhibit, prevent, or destroy the growth of the tumor cells. Such animal hosts include mammalian hosts, including human and non-human primate hosts. The adenoviral vector particles may be administered

systemically, such as, for example, by intravenous, intraarterial, or intraperitoneal administration. Alternatively, the adenoviral vector particles may be administered by direct, nonsystemic injection of the adenoviral vector particles to site of the tumor. In general, the adenoviral vector particles are administered at a multiplicity of infection of from about 5 to about 20. The exact dosage of adenoviral vector particles which is to be administered is dependent upon a variety of factors, including the age, weight, and sex of the patient, and the type and severity of the tumor to be treated.

The adenoviral particles may be administered as part of a preparation containing adenoviral particles in an amount of at least 1×10^7 pfu, and in general not exceeding 1×10^{10} pfu preferably from about 5×10^7 pfu to about 1×10^9 pfu, and more preferably from about 5×10^7 pfu to about 5×10^8 pfu. The adenoviral particles may be administered in combination with a pharmaceutically acceptable carrier in a volume up to 100 ml.

The adenoviral vector particles may be administered in combination with a pharmaceutically acceptable carrier suitable for administration to a patient, such as, for example, a liquid carrier such as a saline solution, protamine sulfate (Elkins-Sinn, Inc., Cherry Hill, N.J.), water, aqueous buffers such as phosphate buffers and Tris buffers, or Polybrene (Sigma Chemical, St. Louis, MO). The selection of a suitable pharmaceutical carrier is deemed to be apparent to those skilled in the art from the teachings contained herein.

In another embodiment, the viral vector is a retroviral vector. Examples of retroviral vectors which may be employed include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, human immunodeficiency virus,

myeloproliferative sarcoma virus, and mammary tumor virus. The vector is generally a replication incompetent retrovirus particle.

Retroviral vectors are useful as agents to mediate retroviral-mediated gene transfer into eukaryotic cells. Retroviral vectors are generally constructed such that the majority of sequences coding for the structural genes of the virus are deleted and replaced by the gene(s) of interest. Most often, the structural genes (*i.e.*, gag, pol, and env), are removed from the retroviral backbone using genetic engineering techniques known in the art. This may include digestion with the appropriate restriction endonuclease or, in some instances, with Bal 31 exonuclease to generate fragments containing appropriate portions of the packaging signal.

These new genes have been incorporated into the proviral backbone in several general ways. The most straightforward constructions are ones in which the structural genes of the retrovirus are replaced by a single gene which then is transcribed under the control of the viral regulatory sequences within the long terminal repeat (LTR). Retroviral vectors have also been constructed which can introduce more than one gene into target cells. Usually, in such vectors one gene is under the regulatory control of the viral LTR, while the second gene is expressed either off a spliced message or is under the regulation of its own, internal promoter. Alternatively, two genes may be expressed from a single promoter by the use of an Internal Ribosome Entry Site.

Efforts have been directed at minimizing the viral component of the viral backbone, largely in an effort to reduce the chance for recombination between the vector and the packaging-defective helper virus within packaging cells. A packaging-defective helper virus is necessary to provide

the structural genes of a retrovirus, which have been deleted from the vector itself.

Examples of retroviral vectors which may be employed include, but are not limited to, Moloney Murine Leukemia Virus vectors such as those described in Miller, *et al.*, Biotechniques, Vol. 7, pgs. 980-990 (1989), and in Miller, *et al.*, Human Gene Therapy, Vol. 1, pgs. 5-14 (1990).

In a preferred embodiment, the retroviral vector may include at least four cloning, or restriction enzyme recognition sites, wherein at least two of the sites have an average frequency of appearance in eukaryotic genes of less than once in 10,000 base pairs; *i.e.*, the restriction product has an average DNA size of at least 10,000 base pairs. Preferred cloning sites are selected from the group consisting of NotI, SnaBI, SalI, and XhoI. In a preferred embodiment, the retroviral vector includes each of these cloning sites. Such vectors are further described in U.S. Patent Application Serial No. 08/340,805, filed November 17, 1994, and in PCT Application No. W091/10728, published July 25, 1991, and incorporated herein by reference in their entireties.

When a retroviral vector including such cloning sites is employed, there may also be provided a shuttle cloning vector which includes at least two cloning sites which are compatible with at least two cloning sites selected from the group consisting of NotI, SnaBI, SalI, and XhoI located on the retroviral vector. The shuttle cloning vector also includes at least one desired gene which is capable of being transferred from the shuttle cloning vector to the retroviral vector.

The shuttle cloning vector may be constructed from a basic "backbone" vector or fragment to which are ligated one or more linkers which include cloning or restriction enzyme recognition sites. Included in the cloning sites are the compatible, or complementary cloning sites hereinabove

described. Genes and/or promoters having ends corresponding to the restriction sites of the shuttle vector may be ligated into the shuttle vector through techniques known in the art.

The shuttle cloning vector can be employed to amplify DNA sequences in prokaryotic systems. The shuttle cloning vector may be prepared from plasmids generally used in prokaryotic systems and in particular in bacteria. Thus, for example, the shuttle cloning vector may be derived from plasmids such as pBR322; pUC 18; etc.

The vector includes one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, *et al.*, Biotechniques, Vol. 7, No. 9, 980-990 (1989), or any other promoter (*e.g.*, cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and ß-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, TK promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The vector then is employed to transduce a packaging cell line to form a producer cell line. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, ψ-2, ψ-AM, PA12, T19-14X, VT-19-17-H2, ψ CRE, ψ CRIP, GP+E-86, GP+envAm12, and DAN cell lines, as described in Miller, Human Gene Therapy, Vol. 1, pgs. 5-14 (1990). The vector containing the polynucleotide encoding wild-type p53 transduces the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation.

The packaging cells thus become producer cells which generate retroviral vectors which include a polynucleotide

encoding wild-type p53. Such retroviral vectors then are transduced into the tumor cells, whereby the transduced tumor cells will express p53.

The retroviral vectors are administered to a host in an amount which in combination with radiation therapy is effective to inhibit, prevent, or destroy the growth of the tumor cells. Such administration may be by systemic administration as hereinabove described, or by direct injection of the retroviral vectors in the tumor. In general, the retroviral vectors are administered in an amount of at least 1×10^7 cfu, and in general, such an amount does not exceed 1×10^8 cfu. Preferably, the retroviral vectors are administered in an amount of from about 2×10^7 cfu to about 5×10^7 cfu. The exact dosage to be administered is dependent upon a variety of factors including those hereinabove described.

The retroviral vectors also may be administered in conjunction with an acceptable pharmaceutical carrier, which may be as hereinabove described.

In another alternative, retroviral producer cells, such as those derived from the packaging cell lines hereinabove described, which include a polynucleotide encoding wild type p53, may be administered to a host. Such producer cells may, in one embodiment, be administered systemically (e.g., intravenously or intraarterially) at a point in close proximity to the tumor, or the producer cells may be administered directly to the tumor. The producer cell line then produces retroviral vectors including a polynucleotide encoding wild type p53 *in vivo*, whereby such retroviral vectors then transduce the tumor cells.

In conjunction with the transduction of the tumor cells with a polynucleotide encoding wild-type p53, radiation also is administered to the tumor cells in an amount effective to inhibit, prevent, or destroy the growth of the tumor cells.

Radiation which may be employed includes, but is not limited to, X-rays, and gamma-rays (photon radiation). Also, charged (*e.g.*, electrons, protons, and heavy ions such as helium and neon) and uncharged particles (*e.g.*, neutrons) may be employed in radiation treatment. In general, the radiation is administered at about the same time or subsequent to the transduction of the tumor cells with a polynucleotide encoding wild-type p53. The radiation may be administered as a single dose or in multiple doses administered at intervals of from about 24 hours to about 48 hours. The total dose of radiation administered may be from about 20 Gy to about 50 Gy, preferably from about 20 Gy to about 25 Gy. Preferably, the radiation is administered in 10 doses in an amount of from about 2.0 Gy to about 2.5 Gy per dose. The exact dosage of radiation to be administered is dependent upon a variety of factors including those hereinabove described, as well as the normal tissue tolerance of the area exposed to the radiation.

Tumors which may be treated in accordance with the present invention include malignant and non-malignant tumors. The tumors may be those which normally are radiation resistant, as well as non-radiation resistant (i.e., radiation-sensitive) tumors.

Malignant (including primary and metastatic) tumors which may be treated include, but are not limited to, cancers which may be found in the oral epithelium, including, but not limited to, squamous cell carcinomas of the mouth, oral cavity, and upper aerodigestive tract, including the floor of the mouth, tongue, cheek, gums, or palate, adenocarcinoma of the oral cavity, lip cancers, Kaposi's sarcoma, and laryngeal papillomas and nasopharyngeal cancers which may have spread to the oral epithelium; tumors occurring in the adrenal glands; bladder, bone; breast; cervix; endocrine glands (including thyroid glands, the pituitary gland, and the pancreas); stomach; small intestine; peritoneal cavity;

colon; rectum; heart; hematopoietic tissue; kidney; liver; lung; muscle; nervous system; brain; eye; oral cavity; pharynx; larynx and other head and neck cancers; ovaries; penis; prostate; skin (including melanoma, basal cell carcinoma, and squamous cell carcinoma); testicles; thymus; and uterus.

The present invention is applicable particularly to the treatment of squamous cell carcinomas of the head and neck, and of the upper aerodigestive tract. For example, in one embodiment, a viral vector, such as an adenoviral vector, is administered to an animal host in an amount of from about 5 x 10^7 pfu to about 5 x 10^8 pfu. At about 36 hours to about 48 hours after injection of the viral vectors, radiation is administered in 8 to 10 doses in an amount of from about 2.0 Gy to about 2.5 Gy per dose.

EXAMPLES

The invention now will be described with respect to the following examples; however, the scope of the present invention is not intended to be limited thereby.

MATERIALS

A. *In Vitro Systems*. One of the cell lines employed is the SCCHN cell line JSQ-3. This line was derived from a tumor of the nasal vestibule which had failed radiotherapy (Weichselbaum, *et al.*, Int. J. Radiation Oncology Biol. Phys., Vol. 15, pgs. 575-579 (1988)). This cell line is characterized as being significantly radiation resistant with a D_0 value of 263 (Weichselbaum, *et al.*, Head and Neck Cancer, Vol. 3, pgs. 399-407 (1993)). More recently, it was shown by Jung, *et al.*, 1992, to carry a mutated form of p53. Two mutations were identified in the cell line, one in exon 4 at codon 72 (G-->C) and one in exon 8 at codon 298 (G-->T) (Jung, *et al.*, 1992). In addition, an activated form of the raf-1 gene was isolated from this cell line (Kasid, *et al.*, Science, Vol. 237, pgs. 1039-1041 (1987)). These cells, therefore, represent a good model to test the effectiveness

of the replacement of wt p53 (via an adenoviral vector) on decreasing radiation resistance.

Other cell lines which are employed include the SQ-20-B cell line, derived from a squamous cell carcinoma of the larynx (Weichselbaum, *et al.*, Proc. Nat. Acad. Sci., Vol. 83, pgs. 2684-2688 (1986)), and SCC 61, derived from a tongue tumor (Weichselbaum, 1986). These cell lines, along with the JSQ-3 cell line and the human non-cancerous skin fibroblast cell line H500, were maintained in Minimum Essential Medium with Earle's salts, supplemented with 10% heat-activated fetal bovine serum; 50 g/ml each of penicillin, streptomycin, and neomycin; 2 mM L-glutamine; 0.1 mM non-essential amino acids and 1 mM sodium pyruvate. Human breast (SK-BR-3) and ovarian (SK-OV-3) carcinoma cell lines (both obtained from ATCC, Rockville, Md.) were maintained in McCoy's 5A medium supplemented with 10% fetal bovine serum, 50 μg/ml each of penicillin, streptomycin, and neomycin, and 2 mM L-glutamine.

B. *In Vivo* Model. A nude mouse model system is employed to determine if the introduction of replicative defective human adenovirus carrying wt p53 (Av1p53) is effective in inhibiting the growth of a xenograft induced by cells derived from a squamous cell carcinoma of the human head and neck (JSQ-3), both alone and in combination with radiation treatment. The model is similar to that described by Clayman *et al.*, Cancer Research, Vol. 55, pgs. 1-6 (1995). In this xenograft model tumor cells are injected subcutaneously on the lower back of the mouse. This system is advantageous in that this system facilitates exposure of the resulting tumor to radiation. Moreover, it is easy to measure tumor size and therefore to assess the effectiveness of the treatment. This model can also be used to mimic the post-surgical microscopic residual environment of SCCHN. With this model, the major difference from the examples contained herein is that in the examples contained herein the

emphasis is placed on the combination of Av1p53 and radiotherapy.

C. Construction of Av1LacZ4 and Av1p53

The adenoviral vectors Av1LacZ4 and Av1p53 are replication deficient E1a/E1b, E3 deletion mutants containing a LacZ (β-galactosidase) gene and a p53 gene, respectively.

Av1LacZ4 was constructed from the adenoviral shuttle vector pAvS6 (Figure 1), which is described in published PCT Application Nos. WO94/23582, published October 27, 1994 and WO95/09654, published April 13, 1995.

The recombinant, replication-deficient adenoviral vector Av1LacZ4, which expresses a nuclear-targetable B-galactosidase enzyme, was constructed in two steps. First, a transcriptional unit consisting of DNA encoding amino acids 1 through 4 of the SV40 T-antigen followed by DNA encoding amino acids 127 through 147 of the SV40 T-antigen (containing the nuclear targeting peptide Pro-Lys-Lys-Lys-Arg-Lys-Val), followed by DNA encoding amino acids 6 through 1021 of E. coli B-galactosidase, was constructed using routine cloning and PCR techniques and placed into the EcoRV site of pAvS6 to yield pAvS6-nlacZ (Figure 2).

The infectious, replication-deficient, Av1LacZ4 was assembled in 293 cells by homologous recombination. To accomplish this, plasmid pAvS6-nLacZ was linearized by cleavage with KpnI. Genomic adenoviral DNA was isolated from purified Ad-dl327 viruses by Hirt extraction, cleaved with ClaI, and the large (approximately 35 kb) fragment was isolated by agarose gel electrophoresis and purified. Ad-dl 327 (Thimmapaya, *et al.*, Cell, Vol. 31, pg. 543 (1983)) is identical to Adenovirus 5 except that an XbaI fragment including bases 28591 to 30474 (or map units 78.5 to 84.7) of the Adenovirus 5 genome, and which is located in the E3 region, has been deleted. The ClaI fragment was used as the backbone for all first generation adenoviral vectors, and the vectors derived from it are known as Av1.

Five micrograms of linearized plasmid DNA (pAvS6n-LacZ) and 2.5 μg of the large ClaI fragment of Ad-d1327 then were mixed and co-transfected into a dish of 293 cells by the calcium phosphate precipitation method. After 16 hours, the cells were overlaid with a 1:1 mixture of 2% Sea Plaque agar and 2x medium and incubated in a humidified, 37°C, 5% CO_2/air environment until plaques appeared (approximately one to two weeks). Plaques were selected and intracellular vector was released into the medium by three cycles of freezing and thawing. The lysate was cleared of cellular debris by centrifugation. The plaque (in 300 μl) was used for a first round of infection of 293 cells, vector release, and clarification as follows:

One 35 mm dish of 293 cells was infected with 100 μl of plaque lysate plus 400 μl of IMEM-2 (IMEM plus 2% FBS, 2mM glutamine (Bio Whittaker 046764)) plus 1.5 ml of IMEM-10 (Improved minimal essential medium (Eagle's) with 2x glutamine plus 10% vol./vol. fetal bovine serum) plus 2mM supplemental glutamine (Bio Whittaker 08063A) and incubated at 37°C for approximately three days until the cytopathic effect, a rounded appearance and "grapelike" clusters, was observed. Cells and supernatant were collected and designated as CVL-A. Av1LacZ4 vector (a schematic of the construction of which is shown in Figure 3) was released by three cycles of freezing and thawing of the CVL-A. Then, a 60 mm dish of 293 cells was infected with 0.5 ml of the CVL-A plus 3 ml of IMEM-10 and incubated for approximately three days as above. Cells and supernatant from this infection then were processed by three freeze/thaw cycles in the same manner. Av1LacZ4 also is described in Yei, *et al.*, Human Gene Therapy, Vol. 5, pgs. 731-744 (1994); Trapnell, Advanced Drug Delivery Reviews, Vol. 12, pgs. 185-199 (1993), and Smith, *et al.*, Nature Genetics, Vol. 5, pgs. 397-402 (December 1993), which are incorporated herein by reference.

The resultant viral stock was titered by plaque assay on 293 cells using a standard protocol involving a 1.5 hour adsorption period in DMEM/2% FBS, followed by washout and agar overlay of the cell monolayer. (Graham, *et al.*, Virology, Vol. 52, pgs. 456-467 (1973)). The absence of wild-type virus was checked by polymerase chain reaction assays of the stock using primers amplifying a 337 bp fragment of the E1 gene. The stock was negative for wild-type adenovirus using this assay.

The virus stock then was frozen at -80°C and stored until used. The virus stock had a titer of 1.5×10^{11} pfu/ml.

Avlp53 was generated from the plasmid pAvS6.p53 (Figure 4), which was constructed from pAvS6. pAvS6 was digested with EcoRV, and the ends were blunted with calf intestinal alkaline phosphatase. The EcoRV digest linearized the plasmid, opening it in the region between the tripartite leader sequence and the poly A sequence. The linear pAvS6 fragment was gel purified. The p53 gene was obtained from plasmid pp53 (Figure 4). Plasmid pp53 was constructed from pBSK-SN3, obtained from PharmaGenics (Allendale, New Jersey), which contains a 1.8 kb XbaI fragment that includes the wild type p53 open reading frame as well as 5' and 3' untranslated regions cloned into the XbaI site of pBluescriptSK (Stratagene, LaJolla, California). pBSK-SN3 was digested with SmaI and partially digested with NcoI to generate a 1,322 bp fragment containing the p53 open reading frame. The fragment was gel purified and ligated into plasmid pBg (described in published PCT Application No. WO91/10728, published July 25, 1991), in place of the β-galactosidase gene between the NcoI and the XhoI sites to yield plasmid pp53. The plasmid pp53 is digested with SmaI and NotI, and a resulting 1.4 kb fragment including the p53 gene is blunt ended at the 5' end with Klenow (The 3' end was blunt ended as a result of the SmaI digest.) and gel purified. The 1.4 kb NotI-SmaI fragment including the p53 gene is ligated to

the EcoRV fragment obtained from pAvS6 to generate pAvS6.p53 (Figure 4).

Av1p53 (Figure 5) then is generated from pAvS6.p53 by linearization of pAvS6.p53 with NotI, followed by homologous recombination with the large ClaI fragment of Ad-dl327 according to the same procedure as hereinabove described with respect to the generation of Av1LacZ4 from pAvS6n-LacZ. Virus was isolated from plaques formed in the 293 cell monolayer. Specifically, pAvS6p53 was linearized with NotI and gel purified. The Ad-dl327 ClaI fragment (2.5 μg) and 5 μg of linearized pAvS6p53 plasmid were introduced into 293 cells by calcium phosphate transfection. Viral DNA from ten plaques was isolated and digested with BglII and ClaI to generate a 1.4 kb p53 fragment. Digested DNA was fractionated on a 1% agarose gel and transferred to nitrocellulose for Southern hybridization with a radiolabeled p53 specific probe. The crude viral lysate from a p53 positive plaque was used to make a large scale preparation of Av1p53 which was purified by CsCl banding using standard methods. This preparation of Av1p53 then was plaque purified twice by limiting dilution in 293 cells. Ten plaques were isolated and amplified on 293 cells to generate crude viral lysate. The viral DNA prepared from each crude viral lysate was used in a PCR reaction to amplify a 1.6 kb fragment containing the p53 gene. One of these p53 positive plaques then was used to make large scale preparations of Av1p53.

Example 1

3×10^4 JSQ-3, SQ-20B, or SCC 61 cells were plated per well in a 24 well tissue culture dish. 24 hours later, at approximately 50% confluency, they were treated with Av1p53 or Av1LacZ4 in doses of 0 (control), 10(5×10^5 pfu), 20, 40, 80, 160, or 320 MOI (1.6×10^7 pfu). Treatment consisted of incubating the cells with the appropriate virus concentration in a volume of 150 μl to 500 μl of PBS for two hours at 37°C with gentle rocking. At the end of the two hours, 2 ml of

media (McCoy's 5A with 10% FBS) was added to the wells without removing the virus. 48 hours after the first virus treatment, a second virus dose was given in the same manner. 4 days after the second virus treatment the cells were fixed and stained with Giemsa Stain. Visual observation of the status of the cells was made with regard to morphology to approximate the amount of cell killing. The visual estimations of the percentage inhibition of the growth of the JSQ-3 cells treated with Av1p53 were as follows:

0 MOI	-	0%
10 MOI	-	10%
20 MOI	-	30%
40 MOI	-	50%
80 MOI	-	90%
160 MOI	-	100%

The visual estimations of the percentage inhibition of the growth of the JSQ-3 cells treated with Av1LacZ4 were as follows:

0 MOI	-	0%
10 MOI	-	0%
20 MOI	-	0%
40 MOI	-	0%
80 MOI	-	10%
160 MOI	-	30%
320 MOI	-	40%

As shown in Figures 6 and 7, significant inhibition of cell growth with doses of Av1p53 as low as 20 MOI was observed in the JSQ-3 cells. These radiation resistant cells, which were derived from a tumor which failed radiotherapy, have been shown to carry a mutant form of p53 (Jung, *et al.*, Cancer Research, Vol. 52, pgs. 6390-6393 (1992)). Vacuolization was observed down to 10 MOI, and by 80 MOI very few live cells were evident (Figure 7); however, the only notable inhibition of cell growth by Av1LacZ4 in this cell line was with doses of 160 and 320 MOI (Figures 6 and 7) and that is still relatively insignificant in comparison to the effect of Av1p53.

Radioresistant cell line SQ-20B, which was also derived from a tumor which failed radiotherapy, exhibits an alteration in the p53 gene expression (Jung, 1992). These cells showed a level of growth inhibition with Av1p53 similar to that observed in JSQ-3 cells (Figure 6); however, more growth inhibition by Av1LacZ4 was observed at high doses in these cells than in the JSQ-3 cells, indicating some non-specific effect in this cell line. In contrast, both the p53 and LacZ containing adenovirus constructs had only minimal effect on the growth of radiosensitive SCCHN cell line SCC 61, and that only at the highest doses (Figure 6). The p53 status of this cell line has not been determined.

More significantly, treatment with either Av1p53 or Av1LacZ4 had no effect on the growth of a normal human fibroblast cell line H500, which has been shown to contain only wild type p53 (Srivastava, *et al.*, Oncogene, Vol. 7, pgs. 987-991 (1992)). Examination under higher magnification, as had been done for JSQ-3 cells, confirmed this finding. The clear area observed at 160 and 320 MOI with Av1p53 in these cells is an artifact which occurred during Giemsa staining. (Figure 6).

Also shown in Figure 6 is the effect of treatment with Av1p53 or Av1LacZ4 on human ovarian and breast carcinoma cell lines SK-OV-3 and SK-BR-3, respectively, both of which have mutant or no p53 (Johnson, et al., Mol. Cell. Biol., Vol. 11, pgs. 1-11 (1991); Elstner, et al., Cancer Research, Vol. 55, pgs 2822-2830 (1995)). The treatment protocol for these cell lines was identical to that used for the SCCHN lines. Both cell lines display a strong response to the replacement of wild-type p53. SK-BR-3 cells display not only growth inhibition, but almost complete cell killing at a dose of Av1p53 as low as 10 MOI. This cell line also was significantly more sensitive to the LacZ containing adenoviral construct. SK-OV-3, while also highly sensitive to Av1p53, showed more specificity in its response. Here,

significant growth inhibition and cell killing with Av1LaZ4 were observed only at the highest doses. These results indicate that the replacement of wild-type 53 is not just specific for SCCHN and may be an effective treatment for various types of cancer.

The replacement of wild-type 53 was shown by these experiments to affect the growth of JSQ-3 cells in a p53 specific manner. As mentioned above, these cells possess both a mutated p53 gene and a radiation resistant phenotype. These cells therefore are an ideal model with which to examine the usefulness of combining wild-type 53 gene therapy with radiotherapy in the treatment of SCCHN.

Example 2

In this experiment, 1.2×10^5 JSQ-3 cells were plated in each well of a 6 well tissue culture dish. 24 hours later they were treated with 5, 10, or 20 MOI of Av1p53 or 20 MOI of Av1LacZ4 in 500 μl of PBS. 24 or 36 hours after virus treatment the cells were trypsinized, counted, exposed to various doses of gamma radiation and replated. 10-14 days later, the plates were fixed and stained with Giemsa stain. Colonies of 50 or more normal looking cells were counted and the percent survival for each radiation dose calculated (number of colonies/cells plated). D_{10} is the radiation dose in Grays (Gy) required to reduce survival to 10%. A D_{10} value of 6.0 Gy indicates significant radiation resistance. Normal radiation sensitive cells usually have D_{10} values of between 3 and 4 Gy. The data, as shown in Figure 8, clearly shows a dose response relationship with both increasing doses of Av1p53 and time. The Av1p53 treated cells are significantly more radiation sensitive than the untreated controls. A greater effect was observed when the cells were exposed to gamma-rays at 36 hours post-virus treatment as compared to 24 hours. With 20 MOI the D_{10} value is reduced from 6.02 Gy to 4.3 Gy, a value much closer to that of non-radioresistant

cells. Conversely, treatment with 20 MOI of Av1LacZ4 has a minimal effect on the survival level of the cells.

These results clearly demonstrated that the restoration of wt p53 is capable of reverting the radiation resistant phenotype of this SCCHN cell line *in vitro*.

Moreover, the large differences in radioresistance levels seen before and after treatment with 20 MOI, and even 10 MOI (D_{10}=4.63 ± 0.12Gy), of Av1p53 are statistically significant ($p < 0.001$), In terms of survival, a decrease of almost 2 Gy represents a dramatic increase in sensitization to the killing effects of ionizing radiation. By comparison, the radiation resistance level of the cells treated with 20 MOI of Av1LacZ4 was reduce only minimally, to a value approximately that observed with only 5 MOI of the p53 construct. Survival curves for the above data are given in Figure 9.

As stated above, the radiation resistance level of the Av1p53 treated cells decreased with increasing time, up to 36 hours after one final treatment. However, by 48 hours post-infection, the D_{10} values began to increase. This rise was inversely proportional to Av1p53 dose, the amount of increase being smaller with increasing MOI (data not shown). Conversely, if two treatments with Av1p53 were given 24 hours apart the decrease in radiation resistance observed at 36 hours was maintained, or even amplified 24 hours after the second round of infection (data not shown). These findings indicate that those cells which had not been transduced with the virus after one treatment continue to grow and maintain their initial radiation resistant phenotype thereby leading to an overall increase in resistance of the culture. A second virus treatment, however, leads to an increased number of affected cells in the population, resulting in increased sensitization to ionizing radiation.

Example 3

JSQ-3 cells were infected with Av1p53 in amounts of 0.5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, or 150 MOI. Thirty-six hours after infection, the cells were trypsinized, pelleted, rinsed with PBS, and lysed in RIPA buffer (1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 30 μg/ml aprotinin and 1 mM sodium orthovanidate in PBS) (Santa Cruz Biotechnology, Inc.). After shearing with a 26 gauge needle, 100 μg/ml PMSF was added, the lysate incubated on ice for 30-60 minutes and centrifuged at 13,000 x g for 20 minutes at 4°C to pellet insoluble material. Protein concentration was determined using the micro-BCA Protein Assay Kit (Pierce Biochemicals).

Forty μg of protein lysate was mixed with an equal volume of 2X protein sample buffer (0.05 M Tris (pH 6.8), 3% SDS, 20% glycerol, 6% 2-mercaptoethanol and 0.001% bromophenol blue), boiled for 5 minutes, loaded on a 10% (4% stacking gel) SDS/polyacrylamide gel, and electrophoresed at 200V for 8 hours. The protein was transferred to a nitrocellulose membrane described in Janat, *et al.*, Mol. and Cell. Diff., Vol. 2, pgs. 241-253 (1994). Preparation of membrane and incubation with the primary and secondary antibodies was performed essentially as described in a protocol supplied by Santa Cruz Biotechnology, Inc., with the exception that incubation with the primary antibody (Anti-p53 antibody Ab-2, Oncogene Research Products) was extended to 2 hours, with wash times of 15 minutes per wash. The washings after addition of the secondary antibody (Anti-mouse IgG-HRP, Santa Cruz Biotechnology, Inc.) also were lengthened to 15 minutes per wash.

Visualization of the protein was accomplished using the ECL Western Blotting Kit (Amersham).

To demonstrate that the virally transduced wild type p53 is being expressed, the level of p53 protein in JSQ-3 cells was examined by Western blot analysis, using the pantropic anti-p53 monoclonal antibody Ab-2, 36 hours post-infection with increasing doses of Av1p53. Thirty-six hours was chosen

based upon the timing of the maximal reversal of radioresistance after one viral treatment as discussed above. As shown in Figure 10, infection with increasing MOI of Av1p53 results in a concomitant increase in expression of the exogenous wild-type 53 (top band), as compared to uninfected JSQ-3 cells (C). An increase is also observed in the lower band, the endogenous mutated p53 band, as viral dose increases. A similar observation was made by Liu *et al.*, Cancer Research, Vol. 54, pgs. 3662-3667 (1994) after treatment of two different SCCHN cell lines with a wild type p53-adenoviral construct under control of the CMV promoter. Data indicates that, at least in murine cell lines, wild-type, but not mutant, p53 is capable of transactivating its own promoter through a sequence nearly identical to the NF-KB-binding site (Deffie, *et al.*, Mol. Cell. Biol., Vol. 13, pgs. 3415-3423 (1993)). This increase in endogenous p53 may therefore be attributable to this transcriptional autoregulation.

These results demonstrate that infection with Av1p53 results in production of immunoreactive p53 protein within the transduced cells in a dose dependent manner.

Example 4

4-6 week old female athymic nude mice were injected subcutaneously on the lower back above the tail with 1 X 10^6 JSQ-3 cells (a radiation resistant head and neck tumor cell line) in 50 ul PBS. Eight days later tumors of approximately 2 X 3 X 1 mm were evident at the injection site. The animals were then divided into three groups, and injected, directly into the tumor, with either 5 MOI (5 x 10^7 pfu) or 10 MOI (1 x 10^8 pfu) of Av1p53 (in 50 ul PBS) or 10 MOI (1 x 10^8 pfu) of the control LacZ vector (Av1LacZ4) in 50 ul PBS. 36-48 hours post injection the tumor area only was exposed to a 1.5 Gy dose of X-rays. The same day a second injection of either Av1p53 or Av1LacZ4 was administered. 36-48 hours later, the tumor site was exposed to a 2.0 Gy dose of radiation.

Thereafter, the animals were given radiation every 48 hours to a total dose of 20 Gy. As a control, one mouse, which had been treated with 10 MOI of Av1p53, received no radiation.

The size of each tumor was measured prior to each radiation treatment and the mean tumor volume plotted against time. At the start of the experiment, the Av1p53 5 MOI group contained 5 animals, the Av1p53 10 MOI group contained 6 animals, and the Av1LacZ4 group had 4 animals. By the end of the experiment, due to death and sacrificing of animals for histology, there were 3, 5, and 3 animals surviving, respectively. As shown in Figure 11, the top two arrows indicate virus injections, while the bottom arrows represent doses of radiation. This experiment indicates that Av1p53, even at 5 MOI, in combination with radiation, was able to inhibit and decrease tumor growth, even 40 days after the end of the radiation treatments. On the other hand, the Av1LacZ4 treated tumors, even in the presence of radiation, did not decrease in size.

Example 5

1 X 10^6 JSQ-3 cells (in 50 ul PBS) were again injected subcutaneously on the lower back, above the tail, of 4-6 week-old female athymic nude mice. The results of this example are shown in Figure 12. In brief, once the tumor has reached approximately 6-8 mm^3 in volume, they were randomly divided into seven groups as described below:

Group 1. Control, without virus infection, without irradiation.

Group 2. LacZ vector, two injections at 10 MOI each, plus irradiation.

Group 3. χ-irradiation, without virus injection.

Group 4. Av1p53, two injections, at 10 MOI each, without irradiation.

Group 5. Av1p53, two injections at 10 MOI each, plus irradiation.

Group 6. Av1p53, two injections at 5 MOI each, plus irradiation.

Group 7. Av1p53, one injection at 10 MOI each, plus irradiation.

Thirty-six to forty-eight hours after virus injection, the tumor site was exposed to 5 Gy of radiation for the first two fractions. Subsequent irradiations were performed every 48 hours for six times to a total dose of 25 Gy. Animals were euthanized and tumors taken for histology prior to injection of virus, 36-48 hours after the first virus injection (prior to irradiation), and after completion of the course of radiation therapy. As a control, tumors were injected with the control viral vector (Av1LacZ4) at 10 MOI with irradiation. The tumor response, growth or inhibition, are monitored and measured as tumor volume using the equation: Cubic Volume = $[L \times (W)^2] / 2$.

The experiment has entered its third week and the full course of radiation has been completed. A clear inhibitory effect of radiation on mean tumor volume is evident. All groups with radiation treatment have maintained the tumor volume either the same or smaller then the original volume when the first dose of adenovirus was injected. Tumors in the two groups (1 and 4) without irradiation increased in size exponentially entering the third week.

Example 6

4 to 6 week old female athymic nude mice were injected subcutaneously on the lower back above the tail with 1.5×10^6 JSQ-3 cells in 50 µl PBS. Five days later tumors of approximately 12 mm^3 were evident at the injection site. The animals were divided into groups (8 mice/group) and injected once directly into the tumor, with either 2.5×10^8, 5×10^8, 1×10^9 or 1.5×10^9 pfu of Av1p53. 1.5×10^9 pfu of the control vector Av1LacZ4 was injected into one group and one group was left untreated. 36 to 48 hours post injection, the tumor area only was exposed to a 2.5 Gy dose of ionizing

radiation. Thereafter, the animals were given 2.5 Gy of radiation every 48 hours to a total dose of 20 Gy. For comparison, a group of untreated mice and mice which had been treated with Av1p53, or Av1LacZ4, received no radiation.

The findings presented in Figures 13A-C demonstrate that the combination of radiation and wild type p53 replacement is markedly more effective in controlling tumor growth than either treatment individually. In the animals treated with radiation alone, the tumors initially regressed (Figure 13A); however, these tumors began to recur by approximately five weeks post-radiation. Similarly, those treated with radiation and a high dose (1.5×10^9 pfu) of the control vector Av1LacZ4 also regressed, and then began to regrow at about seven weeks post-treatment.

In contrast, treatment with the combination of radiation and the adenoviral construct containing wild-type p53 resulted in complete eradication of the tumors, even though only one injection of the adenovirus was employed. Because of the immunogenicity of adenovirus, these results are of great significance with respect to the clinical setting. With the exception of those tumors treated with the lowest dose of Av1p53 (2.5×10^8 pfu) (Figure 13B), these tumors did not recur even five months after the last dose of radiation was administered. This observation is represented in Figure 13B where the plots for Av1p53 at 5×10^8, 1×10^9 and 1.5×10^9 pfu go to zero between days 42 to 65 and remain at zero for the duration of the experiment. This is in striking contrast to the results for 1.5×10^9 pfu of Av1LacZ4 (Figure 13A) and 2.5×10^8 pfu of Av1p53 (Figure 13B). Both groups of tumors of which begin to regrow at about 65 to 72 days post-virus infection (approximately 50 days post-irradiation).

In the unirradiated tumors (Figure 13C), treatment with the three lower doses of Av1p53 alone had only minimal inhibitory effect on tumor growth. Although the highest dose

of Av1p53 (1.5×10^9 pfu) was able to induce significant tumor regression for a prolonged period of time, these tumors also began to recur and reached approximately 50% of their initial volume by 162 days after Av1p53 treatment (Figure 13C). Four separate *in vivo* experiments of this nature have been performed with consistent results.

Example 7

Four to six week old female athymic nude mice were injected subcutaneously on the lower back above the tail with 1.5×10^6 JSQ-3 cells in 50 μl PBS as hereinabove described. The resulting subcutaneous tumors were excised from the animals either before or after treatment with radiation and/or Av1p53 or the control vector Av1LacZ4. The tumors then were fixed in Histochoice (Amresco), sectioned, and stained with hematoxylin and eosin. One tumor was excised before treatment with vector or radiation. A second tumor was excised after treatment with two injections of 1×10^8 pfu of Av1p53 without radiation. A third tumor was excised after treatment with 20 Gy of ionizing radiation, without viral treatment. A fourth tumor was excised after treatment with two injections of 1×10^8 pfu of Av1LacZ4 plus 20 Gy of ionizing radiation. A fifth tumor was excised after treatment with 20 Gy of ionizing radiation plus 2 injections of 5×10^7 pfu of Av1p53. A sixth tumor was excised after treatment with a combination of 20 Gy of ionizing radiation plus 2 injections of 1×10^8 pfu of Av1p53.

Subcutaneous xenograft tumors and surrounding tissues were removed, fixed, sectioned, and stained with hematoxylin and eosin for evaluation of their histological appearance, with or without the combination of Av1p53 and radiation treatment. The control untreated, unirradiated tumor is shown in Figure 14A. The xenograft exhibited characteristics of squamous cell carcinoma, with keratin pearls and desmosomes. Two injections (48 hours apart) with 1×10^8 pfu Av1p53 without radiation was able to induce some

morphological changes as indicated by the condensation of the chromatin leaving empty space (vacuoles) within the confines of the nuclear membrane. Some tumor cells also appeared more differentiated after Av1p53 treatment (Figure 14B). After exposure to 20 Gy, some post-irradiation effects, necrosis and pyknosis, are identified in both the non-virally treated tumor (Figure 14C) and in the Av1LacZ4 (1×10^8 pfu) injected tumor (Figure 14D); however, a number of live tumor cells are still visible. The presence of these live tumor cells would indicate that these tumors had a high probability of recurrence.

A synergistic effect is clearly seen, however, when gene replacement therapy and radiation are combined. Only very few pyknotic cells (mostly cell debris) at the site previously bearing tumor are present after treatment with 5×10^7 pfu of Av1p53 in combination with 20 Gy of radiation (Figure 14E). Even more striking is the appearance of the tumor tissue remaining after two treatments with 1×10^8 pfu of Av1p53 and 20 Gy (Figure 14F). In the very small residual tumor tissue, there are no live tumor cells and only necrotic tissue is present.

The results of these studies suggest a synergistic effect when wild type p53 replacement is used in concert with standard radiotherapy (See Figures 14B, C, and F).

Example 8

Cell Culture and *In Vitro* Viral Infection

JSQ-3 cells as well as human fibroblast cell line H500, were maintained in Minimum Essential Medium with Earle's salts (EMEM), supplemented with 10% heat-activated fetal bovine serum (FBS); 50 μg/ml each of penicillin, streptomycin and neomycin; 2 mM L-glutamine; 0.1 mM non-essential amino acids and 1 mM sodium pyruvate.

For *in vitro* viral infection, the cells, in six well culture plates at approximately 30-50% confluency, were incubated with Av1p53 or Av1LacZ4 diluted to the appropriate

pfu (Plaque Forming Unit) concentration with 1 ml serum free medium, at 37°C with gentle rocking. At the end of two hours, an equal volume of fresh medium with 20% FBS was added to the culture without removal of the virus.

Flow Cytometry

JSQ-3 cells were infected with 30 MOI (Multiplicity of Infection) (approximately 3 x 10^6 pfu) as described above. At 36 hours post-infection, the cells were trypsinized, pelleted, resuspended in fresh medium and exposed to 8.0 Gy of ^{137}Cs gamma-rays at a dose rate of approximately 36 Gy/min in a J.L. Shepherd and Associates Mark I irradiator. Afterward, the cells were diluted and replated. 24 to 48 hours after irradiation, the cell cycle status was assessed by pulsing the cells for 30 minutes with 10 µl BrdUrd and subsequently staining them for replicative DNA synthesis with a fluorescein isothiocyanate (FITC) - conjugated anti-BrdUrd antibody and for DNA content with propidium iodide (PI), essentially according to a protocol supplied by Becton Dickinson Immunocytometry Systems. Flow cytometry was performed using a Becton Dickinson FACScan flow cytometer. The data from 1 x 10^4 cells was collected and analyzed using the CELLFIT analysis software.

To determine the percent of cells in apoptosis, viral infection and irradiation was performed as above. 72 hours post-irradiation the cells were collected, washed with PBS and resuspended in 100 µl of normal saline. As for cell cycle analysis, the cells were fixed in 70% ice-cold ethanol. After fixation however, the cells were pelleted and rehydrated by incubation for 30 minutes at 37°C in Hank's Balanced Salt Solution (GIBCO). After rehydration, they were resuspended for 30 minutes at 37°C in a 5 µg/ml PI solution. DNA content, as indicated by fluorescence intensity, was measured by flow cytometry as above.

Growth of Human Xenograft Tumors *In Vivo*

2×10^6 human SCCHN tumor cells (JSQ-3) in 50 µl PBS were injected subcutaneously on the lower back above the tail of 4 to 6 week old female athymic nude (athymic NCr-nu) mice. When xenografts of the predetermined size were evident at the injection site, the animals were divided into groups. Each group (7 to 9 mice/group) was injected one time, directly into the tumor, with 5×10^8 to 2×10^9 pfu of the Av1p53 or Av1LacZ4 (in 50 µl PBS). Control tumors were not injected. At 36 to 48 hours post injection, the animals were immobilized in a lead chamber which shielded the body except for the tumor area which was exposed to a 2.5 Gy dose of ionizing radiation using a Philips RT 250, 250 KV X-Ray machine using a 0.5 mm Cu filter with a dose rate of 84 cGy/min. Thereafter, the animals were given 2.5 Gy of radiation every 48 hours to a total dose of 20 Gy. For comparison, one group of untreated tumors, as well as tumors which had been injected with Av1p53 or Av1LacZ4, received no irradiation. The size of each tumor was measured prior to viral infection and to each radiation treatment and weekly thereafter.

Restoration of Cell Cycle Control

Lack of functional p53 leads to loss of the G1 block and deregulation of the cell cycle and is likely to play a role in the radiation resistance observed in many tumors carrying abnormal p53. Because replacement of wild type p53 reverses the radiation resistant phenotype of JSQ-3 cells, it was determined if this sensitization correlates with restoration of the G1 block. Therefore, JSQ-3 cells were infected with 30 MOI of Av1p53. 36 hours later, the cells were exposed to 8.0 Gy of ionizing radiation. The percentage of cells in each phase of the cell cycle was determined by FACS analysis 24 to 48 hours post-irradiation. As shown in Figure 15A, replacement of wild type p53 clearly was able to restore the post-irradiation G1 block in these cells as evidenced by the characteristic increase in the percent of cells in G1 and the

decrease in the percent of cells in S phase in those cells infected with Av1p53. Untreated JSQ-3 cells showed no difference in their cell cycle pattern before or after 8 Gy of radiation. The same cell cycle distribution also was evident in JSQ-3 cells infected with Av1p53 but not exposed to γ-radiation.

In contrast, introduction of wild type p53 into a radiosensitive human fibroblast cell line known to possess wild type p53 had no effect on cell cycle control (Figure 15B). As these cells already possess normal functional wild type p53, radiation exposure induces a G1 block in the non-Av1p53 treated cells, as indicated by the large increase in the percentage of cells in G1, and the almost complete lack of S phase cells. Addition of Av1p53 without radiation treatment, however, has no effect upon the percentage of cells in each phase of the cell cycle, the pattern being similar to that of untreated , unirradiated cells. Furthermore, irradiation of the Av1p53 infected cells yields a pattern virtually identical to that of the non-Av1p53 treated , irradiated cells. These results demonstrate that introduction of wild type p53 via Av1p53 has no detrimental effects on cells containing normal wild type p53 with regards to cell cycle control.

Induction of Apoptosis

In addition to loss of the G1 checkpoint, lack of functional p53 results in a decrease in the ability of the cell in initiate programmed cell death (apoptosis) in response to stress of DNA damage induced, for example, by radiation. Replacement of wild type p53 should therefore restore the apoptotic response to irradiation. To examine the effect of wild type p53 restoration on the induction of apoptosis, JSQ-3 cells were infected with 30 MOI of Av1p53 for 36 hours and then exposed to 8.0 Gy of γ radiation. At 72 hours post-irradiation the percent of apoptotic cells in the population was determined by FACS analysis. A lower

fluorescence intensity, due to reduced DNA content, is indicative of apoptotic cells.

As shown in Figure 16, a clear induction in the percent of apoptotic cells after irradiation is evident in the Av1p53 infected JSQ-3 cells relative to uninfected, irradiated cells, with the percentage increasing from 23% to 38%. The low number of apoptotic cells in the control and the virally treated, unirradiated population also demonstrates that the apoptotic pathway is triggered by exposure to ionizing radiation and not by the introduction of p53.

Enhancement of the Effect of Radiotherapy of by Wild-Type p53 Replacement *In Vivo*

The above results have indicated that treatment with Av1p53 was able to affect tumor growth individually and the level of radiation resistance, and cell cycle control/apoptosis *in vitro*. The clinical relevance of this form of gene therapy would be enhanced greatly if synergism between replacement of p53 and radiation therapy could be demonstrated *in vivo*.

To ascertain the effectiveness of the combination therapy, 4 to 6 week old female athymic nude mice were injected subcutaneously on the lower back above the tail with 2×10^6 JSQ-3 cells. Five days later tumors of approximately 18 mm^3 were evident at the injection site. Each group (7 to 9 animals) was injected once, directly into the tumor, with either 5×10^8, 1×10^9, or 2×10^9 pfu of Av1p53. Alternatively, 5×10^8 or 2×10^9 pfu of Av1LacZ4 was injected into one group and one group was left untreated. At 36 to 48 hours post injection, the tumor area only was exposed to a 2.5 Gy dose of ionizing radiation. Thereafter, the animals were given 2.5 Gy of radiation every 48 hours to a total dose of 20 Gy. For comparison, a group of untreated mice and mice which had been treated with Av1p53, or Av1LacZ4 received no radiation.

The findings, presented in Figure 17, demonstrate that the combination of both radiation and virus is clearly more effective in controlling tumor growth than radiation alone. The mean tumor volume of all irradiated groups decreased throughout the course of the radiation treatment and continued to decline until 30 days after the last irradiation (45 days post-virus injection). At this point the tumors treated with just radiation began to recur and rapidly increased in size. Similarly, the tumors treated with radiation and the control viral construct Av1LacZ4 began to regrow approximately 8 weeks post-irradiation (Day 66).

In contrast, of the tumors treated with the combination of Av1p53 and radiation, regrowth was observed only in those receiving the lowest dose of Av1p53 [5 x 10^8 pfu, P5 (+) R] and that was not evident until approximately 11 weeks post-irradiation. No regrowth of the tumors infected with 1 x 10^9 or 2 x 10^9pfu of Av1p53 was evident even 12 weeks after the last dose of radiation had been administered (100 days post-virus infection). At the same time only minimal growth inhibition was evident in the tumors which did not receive radiation treatment, regardless of whether they had been infected with Av1p53,Av1LacZ4, or had received no viral treatment (data not shown). The decrease in the size of the control tumors observed at approximately day 60 was due to necrosis and scabbing of many of the tumors.

The demonstration in vivo of an enhanced therapeutic effect due to the combination of wild type p53 replacement gene therapy and conventional radiotherapy would have great clinical significance. Such an effect may result in a reduction of the dose of radiation required for effective treatment of head and neck cancer and a greatly decreased rate of recurrence after radiotherapy. The sensitization of the tumors to radiation by Av1p53 observed in vitro, and presumably due to the restoration of G1 block and/or apoptosis described here, carried over to the in vivo

situation. The combination of wild type p53 gene therapy and conventional radiotherapy, at a relatively low radiation dose, was clearly more effective than radiotherapy alone. The Av1p53 infected tumors showed no recurrence for at least twelve weeks after the end of therapy. Given the adverse side effects associated with high doses of radiation, radiosensitization of tumors, resulting in a decrease in the amount of radiation necessary to be effective, would be of immense benefit to the patient. Furthermore, restoration of wild type p53 function, not only in primary tumors, but also in tumors which had previously failed radiotherapy, would sensitize these tumors and thus allow further therapeutic intervention.

The disclosure of all patents, publications (including published patent applications), and database accession numbers and depository accession numbers referenced in this specification are specifically incorporated herein by reference in their entirety to the same extent as if each such individual patent, publication, and database accession numbers and depository accession numbers were specifically and individually indicated to be incorporated by reference.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

What Is Claimed Is:

1. A process for improving the treatment of a tumor by radiation therapy comprising:

treating a tumor by radiation therapy wherein cells of said tumor have been transduced with a polynucleotide encoding wild type p53.

2. The process of Claim 1 wherein said polynucleotide encoding wild-type p53 is contained within a viral vector.

3. The process of Claim 2 wherein said viral vector is a DNA virus vector.

4. The process of Claim 3 wherein said DNA virus vector is an adenoviral vector.

5. The process of Claim 4 wherein said adenoviral vector is administered in an amount of from about 1×10^7 pfu to about 1×10^{10} pfu.

6. The process of Claim 5 wherein said adenoviral vector is administered in an amount of from about 5×10^7 pfu to about 1×10^9 pfu.

7. The process of Claim 1 wherein said radiation is gamma radiation.

8. The process of Claim 1 wherein said radiation is in the form of x-rays.

9. The process of Claim 1 wherein said tumor is a squamous cell carcinoma of the head and neck.

10. The process of Claim 1 wherein said radiation is administered in a total amount of from about 20 Gy to about 50 Gy.

11. The process of Claim 10 wherein said radiation is administered in a total amount of from about 20 Gy to about 25 Gy.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15342

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 48/00
US CL :514/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS MEDLINE BIOSIS EMBASE CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LIU ET AL., "Growth Suppression of Human Head and Neck Cancer Cells by the Introduction of a Wild-Type p53 Gene Via a Recombinant Adenovirus", Cancer Research, 15 July 1994, Volume 54, pages 3662-3667, see entire document.	1-11
Y	WILLS ET AL., "Development and Characterization of Recombinant Adenoviruses Encoding Human p53 for Gene Therapy of Cancer", Human Gene Therapy, September 1994, Volume 5, pages 1079-1088, see entire document.	1-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15342

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NABEYA ET AL., "The Mutational Status of p53 Protein in Gastric Cancer Cell Lines Predicts Sensitivity to Chemotherapeutic Agents", Proceedings of the American Association for Cancer Research, March 1994, Volume 35, page 602, see entire abstract.	1-11